

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning at page 4, line 17 with the following amended paragraph.

Figure 1. Nucleotide sequence (SEQ ID NO: 1) and deduced amino acid sequence (SEQ ID NO: 2) for cDNA clone derived from 55 kD TNF-BP. The 19 amino acid transmembrane region is underlined. Hypothetical glycosylation sites are identified by asterisks.

Please replace the paragraph beginning at page 4, line 35 with the following amended paragraph.

Figure 4. Nucleotide sequence (SEQ ID NO: 3) and deduced amino acid sequence (SEQ ID NO: 4) for cDNA clones derived from 75/65 kD TNF-BP.

Please replace the paragraph beginning at page 5, line 11 with the following amended paragraph.

The TNF-binding proteins of the present invention include homogenous proteins containing the amino acid sequence depicted in Figure 1 (SEQ ID NO: 2) or in Figure 4 (SEQ ID NO: 4), proteins containing fragments of either sequence, and analogues of any such proteins for example proteins containing amino acid sequences analogous to the amino acid sequences of Figure 1 (SEQ ID NO: 2) or Figure 4 (SEQ ID NO: 4) or to fragments thereof. An analogue is a protein in which one or more amino acids of the sequences depicted in Figure 1 (SEQ ID NO: 2) or in Figure 4 (SEQ ID NO: 4) have had their side-groups chemically modified in a known manner, or those in which one or more amino acids have been replaced or deleted, without thereby eliminating TNF-binding ability. Such analogues may be produced by known methods of peptide chemistry, or by known methods of recombinant DNA technology, such as planned mutagenesis.

Please replace the paragraph beginning at page 7, line 13 with the following amended paragraph.

In more detail, the proteins of the present invention are non-soluble proteins, i.e. for example membrane proteins or so-called receptors, and soluble or non-soluble

fragments thereof, which bind TNF (TNF-BP), in homogeneous form, as well as their physiologically compatible salts. Preferred proteins are those which according to SDS-PAGE under non-reducing conditions are characterized by apparent molecular weights of about 55 kD, 51 kD, 38 kD, 36 kD and 34 kD or 75 kD and 65 kD, especially those with about 55 kD and 75 kD. Furthermore, there are preferred those proteins which are characterized by containing at least one of the following amino acid partial sequences:

(IA) Leu-Val-Pro-His-Leu-Gly-Asp-Arg-Glu-Lys-Arg-Asp-Ser-Val-Cys-Pro-Gln-Gly-Lys-Tyr-Ile-His-Pro-Gln-X-Asn-Ser-Ile (SEQ ID NO: 5)

(IB) Ser-Thr-Pro-Glu-Lys-Glu-Gly-Glu-Leu-Glu-Gly-Thr-Thr-Thr-Lys (SEQ ID NO: 6)

(IIA) Ser-Gln-Leu-Glu-Thr-Pro-Glu-Thr-Leu-Leu-Gly-Ser-Thr-Glu-Glu-Lys-Pro-Leu (SEQ ID NO: 7)

(IIB) Val-Phe-Cys-Thr (SEQ ID NO: 8)

(IIC) Asn-Gln-Pro-Gln-Ala-Pro-Gly-Val-Glu-Ala-Ser-Gly-Ala-Gly-Glu-Ala (SEQ ID NO: 9)

(IID) Leu-pro-Ala-Gln-Val-Ala-Phe-X-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-Ser-Thr-Cys (SEQ ID NO: 9)

(IIE) Ile-X-Pro-Gly-Phe-Gly-Val-Ala-Tyr-Pro-Ala-Leu-Glu (SEQ ID NO: 11)

(IIF) Leu-Cys-Ala-Pro (SEQ ID NO: 12)

(IIG) Val-Pro-His-Leu-Pro-Ala-Asp (SEQ ID NO: 13)

(IIH) Gly-Ser-Gln-Gly-Pro-Glu-Gln-Gln-X-X-Leu-Ile-X-Ala-Pro (SEQ ID NO: 14)

in which X stands for an amino acid residue which could not be unequivocally determined.

Please replace the paragraph beginning at page 32, line 27 with the following amended paragraph.

1., For the 55 kD band (according to non-reducing SDS-PAGE):

Leu-Val-Pro-His-Leu-Gly-Asp-Arg-Glu-Lys-Arg-Asp-Ser-Val-Cys-Pro-Gln-Gly-Lys-Tyr-Ile-His-Pro-Gln-X-Asn-Ser-Ile (SEQ ID NO: 5),

and

Ser-Thr-Pro-Glu-Lys-Glu-Gly-Glu-Leu-Glu-Gly-Thr-Thr-Thr-Lys (SEQ ID NO: 6)

in which X stands for an amino acid residue which could not be determined,

2., for the 51 kD and 38 kD bands (according to non-reducing SDS-PAGE):

Leu-Val-Pro-His-Leu-Gly-Asp-Arg-Glu (SEQ ID NO: 15)

3., for the 65 kD band (according to non-reducing SDS-PAGE)

In the N-terminal sequencing of the 65 kD band two parallel sequences were determined up to the 15th residue without interruption. Since one of the two sequences corresponded to a partial sequence of ubiquitin [36, 37], the following sequence was derived for the 65 kD band:

Leu-Pro-Ala-Gln-Val-Ala-Phe-X-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-Ser-Thr-Cys. (SEQ ID NO: 16)

in which X stands for an amino acid residue which could not be determined.

Please replace the paragraph beginning at page 33, line 20 with the following amended paragraph.

Additional peptide sequences for 75(65)kDa-TNF-BP were determined:

Ile-X-Pro-Gly-Phe-Gly-Val-Ala-Tyr-Pro-Ala-Leu-Glu (SEQ ID NO: 11)

and

Ser-Gln-Leu-Glu-Thr-Pro-Glu-Thr-Leu-Leu-Gly-Ser-Thr-Glu-Glu-Lys-Pro-Leu (SEQ ID NO: 7) and Val-Phe-Cys-Thr (SEQ ID NO: 8)

and

Asn-Gln-Pro-Gln-Ala-Pro-Gly-Val-Glu-Ala-Ser-Gly-Ala-Gly-Glu-Ala (SEQ ID NO: 9) and Leu-Cys-Ala-Pro (SEQ ID NO: 12)

and

Val-Pro-His-Leu-Pro-Ala-Asp (SEQ ID NO: 13)

and

Gly-Ser-Gln-Gly-Pro-Glu-Gln-Gln-X-X-Leu-Ile-X-Ala-Pro (SEQ ID NO: 14),

in which X stands for an amino acid residue which could not be determined.

Please replace the paragraph beginning at page 34, line 16 with the following amended paragraph.

Starting from the amino acid sequence according to formula IA there were synthesized having regard to the genetic code for the amino acid residues 2-7 and 17-23 corresponding completely degenerated oligonucleotides in suitable complementarity ("sense" and "antisense" oligonucleotides). Total cellular RNA was isolated from HL60 cells [42, 43] and the first cDNA strand was synthesized by oligo-dT priming or by priming with the "antisense" oligonucleotide using a cDNA synthesis kit (RPN 1256, Amersham, Amersham, England) according to the instructions of the manufacturer. This cDNA strand and the two synthesized degenerate "sense" and "anti-sense" oligonucleotides were used in a polymerase chain reaction (PCR, Perkin Elmer Cetus, Norwalk, Conn., USA according to the instructions of the manufacturer) to synthesize as a cDNA fragment the base sequence coding for the amino acid residues 8-16 (formula IA). The base sequence of this cDNA fragment accorded to: 5'-AGGGAGAAGAGAGATAGTGTGTGTC-3' (SEQ ID NO: 16). This cDNA fragment was used as a probe in order to identify according to a known procedure a cDNA clone coding for the 55 kD TNF-BP in a λ gt11-cDNA gene bank from human placenta (42, 43). This clone was then cut according to usual methods from the λ -vector and cloned in the plasmids pUC18 (Pharmacia, Uppsala, Sweden) and pUC19 (Pharmacia, Uppsala, Sweden) and in the M13mp18/M13mp19 bacteriophage (Pharmacia, Uppsala, Sweden) (42, 43). The nucleotide sequence of this cDNA clone was determined using a Sequenase kit (U.S. Biochemical, Cleveland, Ohio, USA) according to the details of the manufacturer. The nucleotide sequence and the amino acid sequence derived therefrom for the 55 kD TNF-BP and its signal peptide (amino acid "-28" to amino acid "O") is given in Figure 1 using the abbreviations for bases such as amino acids usual in the state of the art. From sequence comparisons with other already known receptor protein sequences there can be determined a N-terminal domain containing approximately 180 amino acids and a C-terminal domain containing 220 amino acids which are separated from one another by a transmembrane region of 19 amino acids (underlined in Figure 1) which is typical according to the sequence comparisons. Hypothetical glycosylation sites are characterized in Figure 1 by asterisks above the corresponding amino acid.

Please replace the paragraph beginning at page 36, line 7 with the following amended paragraph.

Vectors starting from the plasmid "pN11" were constructed for the expression in COS cells. The plasmid "pN11" contains the efficient promotor and enhancer of the "major immediate-early" gene of human cytomegalovirus ("HCMV"; Boshart et al., Cell 41, 521-530, 1985). After the promotor there is situated a short DNA sequence which contains several restriction cleavage sites, which are present only once in the plasmid ("polylinker"), inter alia the cleavage sites for HindIII, Ball, BamHI and PvuII (see sequence).

PvuII

5'-AAGCTTGGCCAGGATCCAGCTGACTGACTGATCGCGAGATC-3' (SEQ ID NO: 17)

3'-TTCGAACCGGTCCTAGGTCGACTGACTGACTAGCGCTCTAG-5' (SEQ ID NO: 18)

Please replace the paragraph beginning at page 37, line 14 with the following amended paragraph.

The following procedure was used for the construction of the vector "pK19". A DNA fragment which contained only the cDNA coding for the extracellular part of the 55 kD TNF-BP (amino acids -28 to 182 according to Figure 1) was obtained by PCR technology (Saiki et al., Science 230, 1350-1354, 1985, see also Example 8). The following oligonucleotides were used in order to amplify the cDNA from "pN123" coding for the extracellular part of the 55 kD TNF-BP:

BAMHI

5'-CACAGGGATCCATAGCTGTCTGGCATGGGCCTCTCCAC-3' (SEQ ID NO: 19)

ASP718

3'-CGTGACTCCTGAGTCCGTGGTGTATTATCTCTAGACCA
TGGCCC-5' (SEQ ID NO: 20)

Please replace the paragraph beginning at page 39, line 9 with the following amended paragraph.

The plasmid "pVL941" (Luckow and Summers, 1989, "High Level Expression of Nonfused Foreign Genes with Autographa californica Nuclear Polyhedrosis virus

Expression Vectors", *Virology* 170, 31-39) was used for the expression in a baculovirus expression system and was modified as follows. The single EcoRI restriction cleavage site in "pVL941" was removed by cleaving the plasmid with EcoRI and the projecting 5'-end was filled in with Klenow enzyme. The plasmid pVL941/E obtained therefrom was digested with BamHI and Asp718 and the vector trunk was subsequently isolated from an agarose gel. This fragment was ligated with a synthetic oligonucleotide of the following sequence:

| | | | |
|--------------------------|---------------------|--------|----------------------|
| BamHI | EcoRI | Asp718 | |
| 5' - GATCCAGAATTCATAATAG | | | - 3' (SEQ ID NO: 21) |
| 3' - | GTCTTAAGTATTATCCATG | | - 5' (SEQ ID NO: 22) |

Please replace the paragraph beginning at page 40, line 8 with the following amended paragraph.

The following procedure was used for the construction of the transfer vector "pN119". The 1.3 kb EcoRI/EcoRI fragment of the 55 kD TNF-BP cDNA in the "pUC19" plasmid (see Example 8) was digested with BanI and ligated with the following synthetic oligonucleotide:

| | | |
|---|---------------------------|---------------------|
| BanI | Asp718 | |
| 5'-GCACCACATAATAGAGATCTGGTACCGGGAA-3' (SEQ ID NO: 23) | | |
| 3'- | GTGTATTATCTCTAGACCATGGCCC | -5' (SEQ ID NO: 24) |

Please replace the paragraph beginning at page 42, line 5 with the following amended paragraph.

Analogously to the procedure described in Example 9, the cDNA fragment coding for the extracellular region of the 55 kDa TNF-BP was amplified in a polymerase chain reaction, but now using the following oligonucleotides as the primer:

Oligonucleotide 1:

| | |
|--|--|
| Sst I | |
| 5' -TAC GAG CTC GGC CAT AGC TGT CTG GCA TG -3' (SEQ ID NO: 25) | |

Oligonucleotide 2:

Sst I

5' -ATA GAG CTC TGT GGT GCC TGA GTC CTC AG -3' (SEQ ID NO: 26)

Please insert the sequence listing (12 pages) submitted herewith into the application.